SPLICE VARIANTS OF ONCOGENES

FIELD OF THE INVENTION

The present invention concerns novel nucleic acid sequences, vectors and host cells containing them, amino acid sequences encoded by said sequences, and antibodies reactive with said amino acid sequences, as well as pharmaceutical compositions comprising any of the above. The present invention further concerns methods for screening for candidate activators or deactivators utilizing said amino acid sequences. The invention also concerns diagnostic assays utilizing said sequences.

BACKGROUND OF THE INVENTION

Many genes which are involved with tumors are involved with functions which encourage and promote growth and division of cells. Some tumor-involved cells are expressed only in tumor cells, while others are expressed also in normal cells albeit at lower levels. A specific example of tumor-involved genes are oncogenes, which are muted formed of proto-oncogenes.

Generally, proto-oncogenes code for cellular proteins that relay signals to the cell's nuclei thus stimulating growth. These cellular proteins respond to signals from other cells and the signaling process involves several steps among them binding of growth and proliferation regulating factors to the cell membrane, release of second messenger, and a host of other intermediates, in the cell cytoplasm, and activation in the nucleus of transcription factors which move the cells through their growth cycles.

Proto-oncogenes that code for these various components in the cascade may mutate, thus becoming oncogenes that keep the pathways continuously active regardless of the extracellular signals received by the cell. This may result

in over-production of growth factors, flooding of the cell with replication signals, uncontrolled stimulation of the intermediary pathways and unrestrained cell growth driven by elevated levels of transcription factors.

The activation of a proto-oncogene to express its oncogenic potential may occur due to point mutation, chromosome rearrangement, gene amplification (an increase in the number of copies of normal proto-oncogenes within a cell) and viral insertion resulting in the control of the expression of the proto-oncogene by a more active promoter.

Typically, oncogenes exhibit dominant phenotype at the cellular level, i.e. one copy of an activated oncogene is sufficient to produce its oncogenic effect, a phenomena which is termed *"gain of function"*. There is usually a requirement to have more than one mutation in the proto-oncogene in order to change a normal cell line into neoplasia. The oncogene may be transmitted from generation to generation when a proto-oncogene mutates in the germ line, and since as indicated above usually more than one mutation is required, a single mutation results in a dominantly inherited tumor predisposition.

The detection of oncogene is of major importance in the detection of tumors as well as in the detection of predisposition to a specific kind of tumor, which may result from additional mutations on an already mutated pro-oncogene. Oncogenes are detected by a plurality of methods among them PCR amplification, hybridization, as well as detection of the oncogenic product by various immunoassays. The understanding of the site of activity of the oncogene is of course of a major importance in the designing of a suitable therapeutical model for the treatment of the cancer resulting from the activity of said oncogene.

Alternative splicing (AS) is an important regulatory mechanism in higher eukaryotes (P.A. Sharp, *Cell* 77, 805-8152 (1994). It is thought to be one of the most important mechanisms for differential expression related to tissue or development stage specificity. AS influences also: protein stability, protein clearance as well as tissue and cellular localization As may further alter protein function by increasing or decreasing the functionality,and may further affect post

translational modifications, It is known to play a major role in numerous biological systems, including human antibody responses, and sex determination in *Drosophila*, (S. Stamm, M.Q. Zhang, T.G. Marr and D.M. Helfman, *Nucleic Acids Research* **22**, 1515-1526 (1994); B. Chabot, *Trends Genet.* **12**, 472-478 (1996); R.E. Breitbart, A. Andreadis, B. Nadal-Ginard, *Annual Rev. Biochem.*, **56**, 467-495 (1987); C.W. Smith, J.G. Patton, B. Nadal-Ginard, *Annu. Rev. Genet.*, **27**, 527-577 (1989)).

Until recently it was commonly believed that alternative splicing existed in only a small fraction of genes (about 5%). A recent observation based on literature survey of known genes revises this conservative estimate to as high as an estimate that at least 30% of human genes are alternatively spliced (M.S. Gelfand, I. Dubchak, I. Draluk and M. Zorn, *Nucleic Acids Research* **27**, 301-302 (1999). The importance of the actual frequency of this phenomenon lies not only in the direct impact on the number of proteins created (100,000 human genes, for example, would be translated to a much higher number of proteins), but also in the diversity of functionality derived from the process.

Several mechanisms at different stages may be held responsible for the complexity of higher eukaryote which include: alternative splicing at the transcription level, RNA editing at the post-transcriptional level, and post-translational modifications are the ones characterized to date.

GLOSSARY

In the following description and claims use will be made, at times, with a variety of terms, and the meaning of such terms as they should be construed in accordance with the invention is as follows:

"Tumor-involved genes (TIG)" – genes for which there is some scientific indication linking their function, expression, or change in the level of their expression to tumors. This term does not signify necessarily that the genes cause the tumor (although in some cases this is so) but may also indicate that the genes

are a result of the tumor process, for example, they are activated by other genes which are the cause of the tumor.

"Variant nucleic acid sequence" – the sequence shown in any one of SEQ ID NO: 1 to SEQ ID NO: 36, sequences having at least *90% identity* (see below) to said sequence and *fragments* (see below) of the above sequences of least 20 b.p. long. These sequences are sequences coding for a novel, naturally occurring, alternative splice variants of native and known genes which are *tumor-involved genes (TIG)*. It should be emphasized that the novel variants of the present invention are naturally occurring sequences resulting from alternative splicing of the TIGS and not merely truncated, mutated or fragmented forms of known tumor-involved sequences which are artificially produced.

"Variant product – also referred at times as the "variant protein" or ***"variant polypeptide"*** – is an amino acid sequence encoded by the variant nucleic acid sequence which is a naturally occurring mRNA sequence obtained as a result of alternative splicing. The amino acid sequence may be a peptide, a protein, as well as peptides or proteins having *chemically modified* amino acids (see below) such as a glycopeptide or glycoprotein. The variant products are shown in any one of SEQ ID NO: 37 to SEQ ID NO: 72. The term also includes *homologues* (see below) of said sequences in which one or more amino acids has been added, deleted, *substituted* (see below) or *chemically modified* (see below) as well as *fragments* (see below) of this sequence having at least 10 amino acids.

"Nucleic acid sequence" – a sequence composed of DNA nucleotides, RNA nucleotides or a combination of both types and may includes natural nucleotides, chemically modified nucleotides and synthetic nucleotides.

"Amino acid sequence" – a sequence composed of any one of the 20 naturally appearing amino acids, amino acids which have been *chemically modified* (see below), or composed of synthetic amino acids.

"Fragment of variant nucleic acid sequence" – novel short stretch of nucleic acid sequences of at least 20 b.p., which does not appear as a continuous stretch in the *original nucleic acid sequence* (see below). The fragment may be a sequence which was previously undescribed in the context of the published RNA and which affects the amino acid sequence encoded by the known oncogene. For example, where the variant nucleic includes a sequence which was not included in the original sequence of the oncogene (for example a sequence which was an intron in the original sequence) the fragment may contain said additional sequence. The fragment may also be a region which is not an intron, which was not present in the original sequence of the TIG. For example where the variant lacks a non-terminal region which was present in the original sequence of the TIG. The two stretches of nucleotides spanning this region (upstream and downstream) are brought together by splicing in the variant, but are spaced from each by the spliced out region in the original sequence of the TIG and are thus not continuous in the original sequence. A continuous stretch of nucleic acids comprising said two sparing stretches of nucleotides is not present in the original sequence of the TIG and thus falls under the definition of fragment.

"Fragments of variant products" - novel amino acid sequences coded by the *"fragment of variant nucleic acid sequence"* defined above.

"Homologues of variants" – amino acid sequences of variants in which one or more amino acids has been added, deleted or replaced. The addition, deletion or replacement should be in the regions or adjacent to regions where the variant differs from the *original sequence* (see below) of the TIG.

"Conservative substitution" - refers to the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and high substitution frequencies in homologous proteins found in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix or BLOSUM matrix. [Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution.

"Non-conservative substitution" - refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln.

"Chemically modified" - when referring to the product of the invention, means a product (protein) where at least one of its amino acid resides is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques which are well known in the art. Among the numerous known modifications typical, but not exclusive examples include: acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristlyation, pegylation, prenylation, phosphorylation, ubiqutination, or any similar process.

"Biologically active" - refers to the variant product having some sort of biological activity, for example, some physiologically measurable effect on target cells, molecules or tissues, in particular on cancer cells.

"Immunologically active" defines the capability of a natural, recombinant or synthetic varient product, or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies. Thus, for example, an immunologically active fragment of variant product denotes a fragment which retains some or all of the immunological properties of the variant product, e.g can bind specific anti-variant product antibodies or which can elicit an immune response which will generate such antibodies or cause proliferation of specific immune cells which produce variant.

"Optimal alignment" - is defined as an alignment giving the highest percent identity score. Such alignment can be performed using a variety of commercially available sequence analysis programs, such as the local alignment program LALIGN using a ktup of 1, default parameters and the default PAM. A preferred alignment is the one performed using the CLUSTAL-W program from MacVector (TM), operated with an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM similarity matrix. If a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the percent identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the *"gap"* of the first sequence). In case of alignments of known gene sequences with that of the new variant, the optimal alignment invariably included aligning the identical parts of both sequences together, then keeping apart and unaligned the sections of the sequences that differ one from the other.

"Having at least 90% identity" - with respect to two amino acid or nucleic acid sequences, refers to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 90% amino acid sequence identity means that 90% of the amino acids in two or more optimally aligned polypeptide sequences are identical, however this definition explicitly

excludes sequences which are 100% identical with the original sequence from which the variant of the invention was varied.

"Isolated nucleic acid molecule having an variant nucleic acid sequence" - is a nucleic acid molecule that includes the coding variant nucleic acid sequence. Said isolated nucleic acid molecule may include the variant nucleic acid sequence as an independent insert; may include the variant nucleic acid sequence fused to an additional coding sequences, encoding together a fusion protein in which the variant coding sequence is the dominant coding sequence (for example, the additional coding sequence may code for a signal peptide); the variant nucleic acid sequence may be in combination with non-coding sequences, e.g., introns or control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; or may be a vector in which the variant protein coding sequence is a heterologous.

"Expression vector" - refers to vectors that have the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are known and/or commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

"Deletion" - is a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

"Insertion" or "addition" - is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring sequence.

"Substitution" - replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively. As regards amino acid sequences the substitution may be conservative or non- conservative.

"Antibody" – refers to IgG, IgM, IgD, IgA, or IgG antibody. The definition includes polyclonal antibodies or monoclonal antibodies. This term refers to whole antibodies or fragments of the antibodies comprising the antigen-binding domain of the anti-variant product antibodies, e.g. antibodies without the Fc portion, single chain antibodies, fragments consisting of essentially only the variable, antigen-binding domain of the antibody, etc.

Distinguishing antibody" – an antibody capable of binding to the variant product and not the original amino acid sequence of the tumor-involved gene from which it has been varied, or an antibody capable of binding to the original nucleic acid sequence and not to the variant product.

"Activator" - as used herein, refers to a molecule which mimics the effect of the natural variant product or at times even increases or prolongs the duration of the biological activity of said product, as compared to that induced by the variant product. The mechanism may be by any mechanism known to prolonging activities of biological molecules such as binding to receptors; prolonging the lifetime of the molecules; increasing the activity of the molecules on its target; increasing the affinity of molecules to its receptor; inhibiting degradation or proteolysis of the molecules, or mimicking the biological activity of the variants on their targets, etc. Activators may be polypeptides, nucleic acids, carbohydrates, lipids, or derivatives thereof, or any other molecules which can bind to and activate the variant product.

"Deactivator" or ***("Inhibitor")*** - refers to a molecule which modulates the activity of the variant product in an opposite manner to that of the activator, by

decreasing or shortening the duration of the biological activity of the variant product. This may be done by any mechanism known to deactivate or inhibit biological molecules such as block of the receptor, block of active site, competition on binding site in target, enhancement of degradation, etc. Deactivators may be polypeptides, nucleic acids, carbohydrates, lipids, or derivatives thereof, or any other molecules which bind to and modulate the activity of said product.

"Treating a disease" - refers to administering a therapeutic substance effective to ameliorate symptoms associated with a disease, to lessen the severity or cure the disease, or to prevent the disease from occurring. Typically the disease is cancer.

"***Detection***" – refers to a method of detection of a cancer. This term may refer to detection of a predisposition to cancer as well as for establishing the prognosis of the patient by determining the severity of the disease, i.e. determining in which stage the cancer is.

"Probe" – the variant nucleic acid sequence, or a sequence complementary therewith, when used to detect presence of other similar sequences in a sample. The detection is carried out by identification of hybridization complexes between the probe and the assayed sequence. The probe may be attached to a solid support or to a detectable label.

"Original sequence" – the amino acid or nucleic acid sequence of the tumor-involved gene (TIG) from which the variant of the invention have been varied as a result of alternative slicing. This sequence will also be referred to at times as *"tumor-involved-gene" (TIG)*.

SUMMARY OF THE INVENTION

The present invention is based on the finding of several novel, naturally occurring splice variants, which are naturally occurring sequences obtained by alternative splicing of known genes, the expression of the known genes was reported in scientific literature to be involved with tumors (hereinafter *"tumor-involved genes"* or *"TIGS"*). The above term does not signify that the known genes necessarily caused the tumor (although this may be so), merely that they are involved therewith (i.e. expressed in tumor cells) and this expression may be the result of other effects, for example, as a result of expression of other genes. The novel splice variants of the invention are not merely truncated forms, fragments or mutations of the known tumor-involved genes, but rather novel sequences which naturally occur within the body of individuals, and thus have physiological significance.

The term *"alternative splicing"* in the context of the present invention and claims refers to: intron inclusion, exon exclusion, addition or deletion of terminal sequences in the variant as compared to the original sequences, as well as to the possibility of *"intron retention"*. Intron retention is an intermediate stage in the processing of RNA transcripts, where prior to production of fully processed mRNA the intron (naturally spliced in the original TIG sequence) is retained in the variant. These intermediately processed RNAs may have physiological significance and are also within the scope of the invention.

The novel variant products of the invention may have the same physiological activity as the original tumor-involved peptide from which they have been varied (although perhaps at a different level); may have an opposite physiological activity from the activity featured by the original tumor-involved peptide from which they are varied; may have a completely different, unrelated activity to the activity of the original tumor-involved peptide which they are varied; or alternatively may have no activity at all and this may lead to various diseases or pathological conditions, especially cancer. Both in the case the variant has the same activity as well as the case it has the opposite activity as the original TIG sequence,

it may differ from the TIG in its stability, its clearance rate and rate of degradation its tissue and cellular localization, its ligand specificity, its cellular distribution, its temporal expression pathway, manner for up and down regulation, interaction with other components and in other biological properties not necessarily connected to activity.

The novel variants may also serve for detection purposes, i.e. their presence or level may be cancer, a predisposition to cancer or the stage and aggression of the cancer disease, or alternatively the ratio between the level variants and the level original peptide from which they were varied, or the ratio to other variants (all obtained by alternative splicing from the same original sequence of the tumor-involved gene) may be indicative of the presence of cancer, predisposition to cancer or the stage and aggressiveness of the cancer disease.

For example, for detectional purposes, it is possible to establish differential expression of various variants in various tissues. A certain variant may be expressed mainly in one tissue, while the original sequence (tumor-involved sequence) from which it has been varied, or another variant (obtained by alternative splicing from the same original tumor-involved sequence) may, be expressed mainly in another tissue. Understanding of the distribution of the variants in various tissues may be helpful in basic research, for understanding the physiological function of the original tumor-involved genes from which they have been varied, as well as help in targeting pharmaceuticals or in developing pharmaceuticals, and in establishing more accurate modalities of diagnosis.

The study of the variants may also be helpful in distinguishing various stages in the life cycles of the same type of cells which may also be helpful for development of pharmaceuticals for various cancer stages in which cell cycles is non-normal.

Thus the detection may by determination of the presence or the level of expression of the variant within a specific cell population, comparing said presence or level between various cell types in a tissue, between different tissues and between individuals.

Thus the present invention provides by its first aspect, a novel isolated nucleic acid molecule comprising or consisting of any one of the coding sequence SEQ ID NO: 1 to SEQ ID NO: 36, fragments of said coding sequence having at least 20 nucleic acids (provided that said fragments are continuous stretches of nucleotides not present in the original sequence from which the variant was varied), or a molecule comprising a sequence having at least 90%, identity to SEQ ID NO: 1 to SEQ ID NO: 36, provided that the molecule is not completely identical to the original sequence of the tumor-involved gene from which the variant was varied.

The present invention further provides a protein or polypeptide comprising or consisting of an amino acid sequence encoded by any of the above nucleic acid sequences, termed herein *"variant product"*, for example, an amino acid sequence having the sequence as depicted in any one of SEQ ID NO: 37 to SEQ ID NO: 72, fragments of the above amino acid sequence having a length of at least 10 amino acids coded by the above fragments of the nucleic acid sequences, as well as homologues of the above amino acid sequences in which one or more of the amino acid residues has been substituted (by conservative or non-conservative substitution) added, deleted, or chemically modified.

The deletions, insertions and modifications should be in regions, or adjacent to regions, wherein the variant differs from the original sequence of the tumor-involved gene.

For example, where the variant is different from the original sequence of the tumor-involved gene by addition of a short stretch of 10 amino acids, in the terminal or non- terminal portion of the peptide i.e. inclusion of an exon, the invention also concerns homologues of that variant where the additional short stretch is altered for example, it includes only 8 additional amino acids, includes 13 additional amino acids, or it includes 10 additional amino acids, however some of them being conservative or non-conservative substitutes of the original additional 10 amino acids of the novel variants. In all cases the changes in the homolog, as

compared to the original tumor-involved sequence, are in the same regions where the variant differs from the original sequence, or in regions adjacent to said region.

Another example is where the variant lacks a non-terminal region (for example of 20 amino acids) which is present in the original tumor-involved sequence (due for example to exon exclusion). The homologues may lack in the same region only 17 amino acids or 23 amino acids. Again the deletion is in the same region where the variant lacks a sequence as compared to the original tumor-involved sequence, or in a region adjacent thereto. It should be appreciated that once a man versed in the art's attention is directed to the importance of a specific region, due to the fact that this region differs in the variant as compared to the original sequence of the tumor-involved gene, there is no problem in derivating said specific region by addition to it, deleting from it, or substituting some amino acids in it. Thus homologues of variants which are derivated from the variant by changes (deletion, addition, substitution) only in said region as well as in regions adjacent to it are also a part of the present invention. Generally, if the variant is distinguished from the original sequence of the tumor-involved gene by some sort of physiological activity, then the homolog is distinguished from the original tumor-involved sequence in essentially the same manner.

The present invention further provides nucleic acid molecule comprising or consisting of a sequence which encodes the above amino acid sequences, (including the fragments and homologues of the amino acid sequences). Due to the degenerative nature of the genetic code, a plurality of alternative nucleic acid sequences, beyond those depicted in any one of SEQ ID NO: 1 to SEQ ID NO: 36, can code for the amino acid sequences of the invention. Those alternative nucleic acid sequences which code for the same amino acid sequences as coded by the sequence SEQ ID NO: 1 to SEQ ID NO: 36 (i.e. SEQ ID NO: 37 to SEQ ID NO: 72) are also an aspect of the of the present invention.

The present invention further provides expression vectors and cloning vectors comprising any of the above nucleic acid sequences, as well as host cells transfected by said vectors.

The present invention still further provides pharmaceutical compositions comprising, as an active ingredient, said nucleic acid molecules, said expression vectors, or said protein or polypeptide.

These pharmaceutical compositions are suitable for the treatment of various cancers, which can be ameliorated or cured by raising the level of any one of the variant products of the invention. Typically these pharmaceutical compositions are for the treatment of cancer.

By a second aspect, the present invention provides a nucleic acid molecule comprising or consisting of a non-coding sequence which is complementary to that of any one of SEQ ID NO: 1 to SEQ ID NO: 36, or complementary to a sequence having at least 90% identity to said sequence (with the proviso added above) or a fragment of said two sequences (according to the above definition of fragment). The complementary sequence may be a DNA sequence which hybridizes with any one of SEQ of ID NO: 1 to SEQ ID NO: 36 or hybridizes to a portion of that sequence having a length sufficient to inhibit the transcription of the complementary sequence. The complementary sequence may be a DNA sequence which can be transcribed into an mRNA being an antisense to the mRNA transcribed from any one of SEQ ID NO: 1 to SEQ ID NO: 36 or into an mRNA which is an antisense to a fragment of the mRNA transcribed from any one of SEQ ID NO: 1 to SEQ ID NO: 36 which has a length sufficient to hybridize with the mRNA transcribed from SEQ ID NO: 1 to SEQ ID NO: 36, so as to inhibit its translation. The complementary sequence may also be the mRNA or the fragment of the mRNA itself.

The nucleic acids of the second aspect of the invention may be used for therapeutic or diagnostic applications for example as probes used for the detection of the variants of the invention. The presence of the variant transcript or the level of the variant transcript may be indicative of cancer, predisposition to cancer or the stage or aggressiveness of the cancer disease. In addition or alternatively, the ratio of the level of the transcripts of the variants of the invention may also be compared to that of the transcripts of the original sequences of the oncogenes from which

have been varied, or to the level of transcript of other variants (especially obtained by alternative splicing from the same original sequence), and said ratio may be indicative of cancer, predisposition to cancer or the stage or aggressiveness of the cancer disease

The present invention also provides expression vectors comprising any one of the above defined complementary nucleic acid sequences and host cells transfected with said nucleic acid sequences or vectors, being complementary to those specified in the first aspect of the invention.

The invention also provides anti-variant product antibodies, namely antibodies directed against the variant product which specifically bind to said variant product. Said antibodies are useful both for diagnostic and therapeutic purposes. For example said antibody may be as an active ingredient in a pharmaceutical composition as will be explained below.

The present invention also provides pharmaceutical compositions comprising, as an active ingredient, the nucleic acid molecules which comprise or consist of said complementary sequences, or of a vector comprising said complementary sequences. The pharmaceutical composition thus provides pharmaceutical compositions comprising, as an active ingredient, said anti-variant product antibodies.

The pharmaceutical compositions comprising said anti-variant product antibodies or the nucleic acid molecule comprising said complementary sequence, are suitable for the treatment of diseases and pathological conditions where a therapeutically beneficial effect may be achieved by neutralizing the variant (either at the transcript or product level) or decreasing the amount of the variant product or blocking its binding to its target, for example, by the neutralizing effect of the antibodies, or by the effect of the antisense mRNA in decreasing the expression level of the variant sequence. In particular these diseases are cancer diseases and the treatment may also be for amelioration of cancer or for prevention of cancer purposes.

According to the third aspect of the invention the present invention provides methods for detecting the level of the transcript (mRNA) of said variant product in a body fluid sample, or in a specific tissue sample, for example by use of probes comprising or consisting of said coding sequences; or by use of suitable primers in conjunction with suitable amplification techniques; as well as methods for detecting levels of expression of said product in tissue, e.g. by the use of antibodies capable of specifically reacting with the variant products of the invention. Detection of the level of the expression of the variant of the invention in particular as compared to that of the original tumor-involved gene sequence from which it was varied or compared to other variant sequences all varied from the same original TIG sequence may be indicative of a cancer, predisposition to cancer or the stage or aggressiveness of the cancer disease

The method, according to this latter aspect, for detection of a nucleic acid sequence which encodes the variant product in a biological sample, comprises the steps of:

(a) providing a probe comprising at least one of the nucleic acid sequences defined above;

(b) contacting the biological sample with said probe under conditions allowing hybridization of nucleic acid sequences thereby enabling formation of hybridization complexes;

(c) detecting hybridization complexes, wherein the presence of the complexes indicates the presence of nucleic acid sequence encoding the variant product in the biological sample.

The method as described above is qualitative, i.e. indicates whether the transcript is present in or absent from the sample. The method can also be quantitative, by determining the level of hybridization complexes and then calibrating said levels to determining levels of transcripts of the desired variant in the sample.

Both qualitative and quantitative determination methods can be used for diagnostic, prognostic and therapy planning purposes, especially in conjunction

with cancer diseases. In addition qualitative determination may be indicative of the cancer stage.

By a preferred embodiment the probe is part of a nucleic acid chip used for detection purposes, i.e. the probe is a part of an array of probes each present in a known location on a solid support.

The nucleic acid sequence used in the above method may be a DNA sequence an RNA sequence, etc; it may be a coding or a sequence or a sequence complementary thereto (for respective detection of RNA transcripts or coding-DNA sequences). By quantization of the level of hybridization complexes and calibrating the quantified results it is possible also to detect the level of the transcript in the sample.

The present invention further concerns a method for detecting of the nucleic acid sequences which encodes the product in a biological sample comprising the steps of:

(i) contacting the sample primer for amplifying with any one of the nucleic acid sequences of SEQ ID NO: 1 to SEQ ID NO: 36;

(ii) contacting the sample with reagents for nucleic acid amplification and under conditions enabling nucleic acid amplification;

(iii) detecting the presence of amplified sequences, said presence correlates with the presence of any one of SEQ ID NO: 1 to SEQ ID NO: 36 in the sample.

The method may also be quantitative by calibrating the amounts of amplified products.

Methods for detecting mutations in the region coding for the variant product are also provided, which may be methods carried-out in a binary fashion, namely merely detecting whether there is any mismatches between the normal variant nucleic acid sequence of the invention and the one present in the sample, or carried-out by specifically detecting the nature and location of the mutation. Detection of mutations may be of importance in the determination of predisposition to cancer, as well as in attempts to establish the prognosis of the cancer disease.

The present invention also concerns a method for detecting variant product in a biological sample, comprising the steps of:

(a) contacting with said biological sample the antibody of the invention, thereby forming an antibody-antigen complex; and

(b) detecting said antibody-antigen complex

wherein the presence of said antibody-antigen complex correlates with the presence of variant product in said biological sample.

Many diseases are diagnosed by detecting the presence of antibodies against a protein characterizing the disease in the blood, serum or any other body fluid of the patient. The present invention also concerns a method for detecting anti-variant antibody in a biological sample, comprising:

(a) contacting said sample with the variant product of the invention, thereby forming an antibody-antigen complex; and

(b) detecting said antibody-antigen complex

wherein the presence of said antibody-antigen complex correlates with the presence of anti-variant antibody in the sample.

As indicated above, both methods (for detection of variant product and for detection of the anti-variant antibody) can be quantitized to determine the level or the amount of the variant or antibody in the sample, alone or in comparison to the level of the original amino acid tumor-involved sequence from which it was varied or compared to the level of antibodies against the original amino acid sequence, and qualitative and quantitative results may be used for diagnostic, prognostic and therapy planning purposes.

The invention also concerns distinguishing antibodies, i.e. antibodies capable of binding either to the variant product or to the original tumor-involved gene sequence from which the variant has been varied, while not binding to the original sequence or the variant product respectively. These distinguishing antibodies may be used for detection purposes.

By yet another aspect the invention also provides a method for identifying candidate compounds capable of binding to the variant product and modulating its activity (being either activators or deactivators). The method includes:

(i) providing a protein or polypeptide comprising an amino acid sequence substantially as depicted in any one of SEQ ID NO: 37 to 72, or a fragment of such a sequence;

(ii) contacting a candidate compound with said amino acid sequence;

(iii) measuring the physiological effect of said candidate compound on the activity of the amino acid sequences and selecting those compounds which show a significant effect on said physiological activity.

The present invention also concerns compounds identified by the above methods described above, which compound may either be an activator of the variant product or a deactivator thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is a comparison between the amino acid sequence of SEQ ID NO: 37 and the original tumor-involved sequence from which it has been varied;

Fig. 2 is a comparison between the amino acid sequence of SEQ ID NO: 38 and the original tumor-involved sequence from which it has been varied;

Fig. 3 is a comparison between the amino acid sequence of SEQ ID NO: 39 and the original tumor-involved sequence from which it has been varied;

Fig. 4 is a comparison between the amino acid sequence of SEQ ID NO: 40 and the original tumor-involved sequence from which it has been varied;

Fig. 5 is a comparison between the amino acid sequence of SEQ ID NO: 41 and the original tumor-involved sequence from which it has been varied;

Fig. 6 is a comparison between the amino acid sequence of SEQ ID NO: 42 and the original tumor-involved sequence from which it has been varied;

Fig. 7 is a comparison between the amino acid sequence of SEQ ID NO: 43 and the original tumor-involved sequence from which it has been varied;

Fig. 8 is a comparison between the amino acid sequence of SEQ ID NO: 44 and the original tumor-involved sequence from which it has been varied;

Fig. 9 is a comparison between the amino acid sequence of SEQ ID NO: 45 and the original tumor-involved sequence from which it has been varied;

Fig. 10 is a comparison between the amino acid sequence of SEQ ID NO: 46 and the original tumor-involved sequence from which it has been varied;

Fig. 11 is a comparison between the amino acid sequence of SEQ ID NO: 47 and the original tumor-involved sequence from which it has been varied;

Fig. 12 is a comparison between the amino acid sequence of SEQ ID NO: 48 and the original tumor-involved sequence from which it has been varied;

Fig. 13 is a comparison between the amino acid sequence of SEQ ID NO: 49 and the original tumor-involved sequence from which it has been varied;

Fig. 14 is a comparison between the amino acid sequence of SEQ ID NO: 50 and the original tumor-involved sequence from which it has been varied;

Fig. 15 is a comparison between the amino acid sequence of SEQ ID NO: 51 and the original tumor-involved sequence from which it has been varied;

Fig. 16 is a comparison between the amino acid sequence of SEQ ID NO: 52 and the original tumor-involved sequence from which it has been varied;

Fig. 17 is a comparison between the amino acid sequence of SEQ ID NO: 53 and the original tumor-involved sequence from which it has been varied;

Fig. 18 is a comparison between the amino acid sequence of SEQ ID NO: 54 and the original tumor-involved sequence from which it has been varied;

Fig. 19 is a comparison between the amino acid sequence of SEQ ID NO: 55 and the original tumor-involved sequence from which it has been varied;

Fig. 20 is a comparison between the amino acid sequence of SEQ ID NO: 56 and the original tumor-involved sequence from which it has been varied;

Fig. 21 is a comparison between the amino acid sequence of SEQ ID NO: 57 and the original tumor-involved sequence from which it has been varied;

Fig. 22 is a comparison between the amino acid sequence of SEQ ID NO: 58 and the original tumor-involved sequence from which it has been varied;

Fig. 23 is a comparison between the amino acid sequence of SEQ ID NO: 59 and the original tumor-involved sequence from which it has been varied;

Fig. 24 is a comparison between the amino acid sequence of SEQ ID NO: 60 and the original tumor-involved sequence from which it has been varied;

Fig. 25 is a comparison between the amino acid sequence of SEQ ID NO: 61 and the original tumor-involved sequence from which it has been varied;

Fig. 26 is a comparison between the amino acid sequence of SEQ ID NO: 62 and the original tumor-involved sequence from which it has been varied;

Fig. 27 is a comparison between the amino acid sequence of SEQ ID NO: 63 and the original tumor-involved sequence from which it has been varied;

Fig. 28 is a comparison between the amino acid sequence of SEQ ID NO: 64 and the original tumor-involved sequence from which it has been varied;

Fig. 29 is a comparison between the amino acid sequence of SEQ ID NO: 65 and the original tumor-involved sequence from which it has been varied;

Fig. 30 is a comparison between the amino acid sequence of SEQ ID NO: 66 and the original tumor-involved sequence from which it has been varied;

Fig. 31 is a comparison between the amino acid sequence of SEQ ID NO: 67 and the original tumor-involved sequence from which it has been varied;

Fig. 32 is a comparison between the amino acid sequence of SEQ ID NO: 68 and the original tumor-involved sequence from which it has been varied;

Fig. 33 is a comparison between the amino acid sequence of SEQ ID NO: 69 and the original tumor-involved sequence from which it has been varied;

Fig. 34 is a comparison between the amino acid sequence of SEQ ID NO: 70 and the original tumor-involved sequence from which it has been varied;

Fig. 35 is a comparison between the amino acid sequence of SEQ ID NO: 71 and the original tumor-involved sequence from which it has been varied;

Fig. 36 is a comparison between the amino acid sequence of SEQ ID NO: 72 and the original tumor-involved sequence from which it has been varied.

DETAILED DESCRIPTION OF THE INVENTION

Example I: Comparison of variants with original sequences

Original sequences of tumor-involved genes were obtained from GenBank Version 115. Their tumor involvement was determined by comparison between the original sequences and the noval variant sequences was made using the BestFit application from the GCG suite version 10.0 (January 1999), with the defalut values:

Gap creation penalty (GapWeight): 50

Gap extension penalty (GapLengthWeight): 3

The comparison is shown in Fig. 1 to 34 which show the comparison of each of the variant products depicted in SEQ ID NO: 37 to 72 with the original tumor-involved sequence from which it was varied.

The following is a list which gives the name and the description of each original tumor-involved sequence from which the alternative splice variant has been varied by alternative splicing. The description is followed by the internal reference to the novel variant (NV-... etc.) and a short comparison between the variant and the original tumor-involved sequence. It should be noticed that several splice variants may have been originated from the same parent sequence by several different alternative splicings. The following table summarizes the accession number of the original sequence, the terminology of the new variant (NV-1 to NV-34) and the description of the difference between the new variant and the original sequence.

Table

Accession	New variant #	Description of the new variant
KU70_HUMAN	NV-1	The new variant has an alternative 3' exon of 5 aa instead of 240 amino acids. It is probably missing the PHOSPHORYLATION (BY NUCLEAR KINASE NII) site and half of the PRO-RICH domain but retains the LEUCINE-ZIPPER domain.

KU70_HUMAN	NV-2	The new variant has a deletion of 210 aa between residues 304 – 515. Lacks the Pro-rich domain but retains the LEUCINE-ZIPPER domain and PHOSPHORYLATION (BY NUCLEAR KINASE NII) site.
LCK_HUMAN	NV-3	The new variant has an alternative 3' exon of 45 amino acids instead of 163 amino acids. The new variant retains both SH domains and most of the PROTEIN KINASE domain including two ATP BINDING sites and the ACTIVE SITE. It is missing the 3' end of the PROTEIN KINASE domain and lacks the AUTO-PHOSPHORYLATION and PHOSPHORYLATION sites.
LCK_HUMAN	NV-4	Insertion of 58 amino acids after amino acid 62 (insertion does not result in truncation). Insertion in first SH2 domain. The new variant retains all important sites including: the PROTEIN KINASE DOMAIN with two ATP BINDING sites, an ACTIVE SITE and an AUTO PHOSPHORYLATION site. An additional PHOSPHORYLATION site.
OSTP_HUMAN	NV-5	The new variant has an alternative 3' exon of 12 aa instead of 134 aa. The new variant maintains the CELL ATTACHMENT SITE and two GLYCOSILATION sites.
GA45_HUMAN	NV-6	The new variant has an alternative 5' exon of 72 amino acids instead of 125 amino acids. The new variant has a signal peptide and has the two PHOSPHORYLATION (BY CK2) sites.
WN11_HUMAN	NV-7	The new variant has a deletion of 22 amino acids after residue 312 (between 312-334). The new variant has all five potential GLYCOSILATION sites.

WN11_HUMAN	NV-8	The new variant has a deletion of 117 amino acids after residue 116 (between 116-233). The new variant is missing one potential GLYCOSILATION site (out of 5 sites).
KPCT_HUMAN	NV-9	The new variant has an alternative 3' exon of 3 amino acids instead of 94 amino acids. The alternative region is in the protein kinase domain. The new variant maintains the two PHORBOL-ESTER AND DAG BINDING domains, the two ATP binding sites and the active site of the kinase domain.
IRF1_HUMAN	NV-10	The new variant has an alternative 3' exon of 7 amino acids instead of 40 amino acids. The new variant maintains the DNA binding domain.
FGR1_HUMAN	NV-11	The new variant has an alternative 3' exon of 14 amino acids instead of 134 amino acids. The new variant has the entire extracellular domain and the TM, it is missing part of the cytoplasmic domain. The new variant maintains all 3 IMMUNO-GLOBULIN-LIKE DOMAINS, the protein KINASE domain, the ACTIVE site, and the 2 ATP binding sites, but it might be missing one of the two PHOSPHORYLATION (AUTO-) sites.
APE1_HUMAN	NV-12	The new variant has a gap of 22 amino acids between residues 146 – 169. The new variant maintains the active site and site important for substrate recognition.
APE1_HUMAN	NV-13	The new variant has an insertion of 25 amino acids after residue 18. The new variant maintains the active site and site important for substrate recognition.

MAD3_HUMAN	NV-14	The new variant has an alternative 3' exon of 3 amino acids instead of 15 amino acids. It retains all five ANK motifs and the two PHOSPHORYLATION sites.
MAD3_HUMAN	NV-15	The new variant has a deletion of 28 amino acids between 183 – 212. The deletion is in the ANK MOTIF 4. The new variant maintains 4 out of the five ANK MOTIFs and the two PHOSPHORYLATION sites.
EPA4_HUMAN	NV-16	Deletion of 65aa after residue 832 (832-898). Deletion in end of CYTOPLASMIC domain. The 3' end of the PROTEIN KINASE domain is missing, but all important sites are maintained. The new variant has two FYBRONECTIN TYPE III domains and the protein KINASE domain with 2 ATP binding sites, an ACTIVE site and an auto PHOSPHORYLATION site.
ETS2_HUMAN	NV-17	The new variant has a deletion of 26 aa between 87 - 114. The new variant maintains the DNA binding domain.
WN5A_HUMAN 1.	NV-18	The new variant has an alternative 3' exon of 4 amino acids instead of 109. It is identical to the known protein until residue 256. Two GLYCOSILATION sites out of four are missing in the new variant.
TYO3_HUMAN	NV-19	The new variant has an alternative 3' exon of 45 amino acids instead of 216 amino acids. The new variant is missing part of the PROTEIN KINASE domain and its AUTOPHOSPHORYLATION site. However, it maintains all other necessary domains: the ACTIVE site and the two ATP binding sites. The variant retains all 6 GLYCOSILATION sites, the 2 IG-like domains and the 2 FIBRONEXTIN TYPE III domains.

CAD2_HUMAN	NV-20	The new variant has an alternative 3' exon of 10 amino acids instead of 68 amino acids. The new variant maintains the extracellular domain and the TM domain. It is missing the end of the cytoplasmic domain and the SER-RICH domain. However, it has all other necessary domains including :5 CADHERIN REPEATS with 7 GLYCOSILATION sites.
MXI1_HUMAN	NV-21	NV_1 m85527_3 Insertion of 24aa after residue 79. Most likely truncated in insertion. Has basic DNA binding domain, but lacks helix-loop-helix.
MXI1_HUMAN	NV-22	NV_2 m85527_5 Alternative 5' exon. Identical to known from aa 26 to the end. Has a 5' exon of 31 aa versus 25 aa of the known. Has both DNA binding domain and helix loop helix. The alternative 5' exon bares a clathrin repeat. Supported by 4 ests.
MPK3_HUMAN	NV-23	Similar to known RNA at first 290 aa. Alternative 3' exon of 10 aa instead of 28 The new variant maintains the PROTEIN KINASE domain with its two ATP binding sites, the ACTIVE site and two POSPHORYLATION sites.
XRC1_HUMAN	NV-24	First 242aa identical to known RNA. Alternative short 3' exon of 50 aa Instead of 391aa.
XRC1_HUMAN	NV-25	Identical to known RNA in first 241 aa. Alternative 3' exon of 25 aa instead of 392.
XRC1_HUMAN	NV-26	Identical to known RNA in first 186 aa. Alternative short 3' exon of length 61 aa, instead of 447 aa.
XRC1_HUMAN	NV-27	Identical to known RNA in first 540 aa. Alternative 3' exon of 84 aa instead of 93 aa.

MERL_HUMAN	NV-28	Deletion of 29 aa from position 333. The new variant retains the Band 4-1 like domain. (Band 4.1, which links the spectrin-actin cytoskeleton of erythrocytes to the plasma membrane.)
DP1_HUMAN	NV-29	Alternative 3' exon of 21 amino acids instead of 72 amino acids. The new variant retains the two transmembrane domains.
MDR1_HUMAN	NV-30	Alternative exon at 3' end at cytoplasmic domain. 1 aa instead of 3 of the known. Identical to known until aa 1277.
MDR1_HUMAN	NV-31	The new variant is a truncated protein. It has an alternative 3' exon of 12 amino acids instead of 713. It is identical to the known protein until residue 567. The new variant retains only one out of two ATP binding sites, and six out of twelve TM domains. It has one out of three cytoplasmic domains and is truncated in the middle of the second cytoplasmic domain.
MK08_HUMAN	NV-32	Identical to known until aa 205. Truncated. Has additional 13 aa. Lacks part of the protein kinase domain. Retains the active site the two ATP binding sites and the two phosphorylation sites.
MK08_HUMAN	NV-33	Alternative 3' exon of 14 aa instead of 134 aa. Identical to known until residue 293. Lacks end of protein kinase domain. Retains the active site, the two ATP binding sites and the two phosphorylation sites.
MK08_HUMAN	NV-34	Alternative 3' exon of 7 aa instead of 95 aa. Identical to known until residue 332. Has entire protein kinase domain including the active site, the two ATP binding sites and the two phosphorylation sites.

MAPK12_HUMAN	NV-35	The new variant contains 152 N-terminal amino acids of the original protein. The new variant has alternative 25 amino acids in its C-terminus, instead of original 215 amino acids. It contains the NP_BIND (between the amino acids 33 – 41, and the ATP binding site at position 56. The truncated variant has only part of the kinase domain, it lacks the active site and both the phosphorylation sites that activates the kinase. This truncated splice variant can act as dominant negative.
KPCT_HUMAN	NV-36	The new variant has an alternative 3' exon of 36 amino acids instead of 94 original amino acids. The alternative region is in the PROTEIN KINASE domain. The new variant maintains the two PHORBOL-ESTER AND DAG BINDING domains, the two ATP binding sites and the ACTIVE of the KINASE domain.

The following is a list of the original tumor-involved sequences, followed by all the splice variants obtained therefrom (each SEQ ID No. referred to as NV_ and the number of the sequence with a list of differences between the original TIG sequence and the variant.

KU (p70/p80)

KU70_HUMAN

FUNCTION: SINGLE STRANDED DNA-DEPENDENT ATP-DEPENDENT HELICASE. HAS A ROLE IN CHROMOSOME TRANSLOCATION. THE DNA HELICASE II COMPLEX BINDS PREFERENTIALLY TO FORK-LIKE ENDS OF DOUBLE-STRANDED DNA IN A CELL CYCLE-DEPENDENT MANNER. IT WORKS IN THE 3'-5' DIRECTION. BINDING TO DNA MAY BE MEDIATED BY P70.

SUBUNIT: HETERODIMER OF A 70 KD AND A 80 KD SUBUNIT.
SUBCELLULAR LOCATION: NUCLEAR.
PTM: PHOSPHORYLATED IN VIVO AT SERINE RESIDUES (BY SIMILARITY).
DISEASE: INDIVIDUALS WITH SLE AND RELATED DISORDERS PRODUCE EXTREMELY LARGE AMOUNTS OF AUTOANTIBODIES TO P70 AND P86. EXISTENCE OF A MAJOR AUTOANTIGENIC EPITOPE OR EPITOPES ON THE CARBOXY TERMINAL 190 AMINO ACIDS OF P70 CONTAINING THE LEUCINE REPEAT. THE MAJORITY OF AUTOANTIBODIES TO P70 IN MOST SERA FROM PATIENTS WITH SLE SEEM TO BE REACTIVE WITH THIS REGION.
SIMILARITY: BELONGS TO THE ATP-DEPENDENT DNA HELICASE II 70 KD SUBUNIT FAMILY.

NV_1

The new variant has an alternative 3' exon of 5 amino acids instead of 240 amino acids. It is probably missing the PHOSPHORYLATION (BY NUCLEAR KINASE NII) site and half of the PRO-RICH domain but retains the LEUCINE-ZIPPER domain.

KU (p70/p80)

KU70_HUMAN

NV_2

The new variant has a deletion of 210 amino acids between residues 304 – 515. The new variant lacks the PRO-RICH domain but retains the LEUCINE-ZIPPER domain and PHOSPHORYLATION (BY NUCLEAR KINASE NII) site.

LCK

LCK_HUMAN

PROTO-ONCOGENE TYROSINE-PROTEIN KINASE LCK

FUNCTION: MAY PARTICIPATE IN ANTIGEN-INDUCED T-CELL ACTIVATION.
CATALYTIC ACTIVITY: ATP + A PROTEIN TYROSINE = ADP + PROTEIN TYROSINE PHOSPHATE.
ENZYME REGULATION: REGULATED BY PHOSPHORYLATION ON TYR-504.
SUBCELLULAR LOCATION: BOUND TO THE CYTOPLASMIC DOMAIN OF EITHER CD4 OR CD8.
SIMILARITY: CONTAINS 1 SH2 DOMAIN.
SIMILARITY: CONTAINS 1 SH3 DOMAIN.
SIMILARITY: TO OTHER PROTEIN-TYROSINE KINASES IN THE CATALYTIC DOMAIN. BELONGS TO THE SRC SUBFAMILY.

NV_3

The new variant has an alternative 3' exon of 45 amino acids instead of 163 amino acids. The new variant retains both SH domains and most of the PROTEIN KINASE domain including two ATP BINDING sites and the ACTIVE SITE. It is missing the 3' end of the PROTEIN KINASE domain and lacks the AUTO-PHOSPHORYLATION and PHOSPHORYLATION sites.

LCK

LCK_HUMAN

NV_4

Insertion of 58 amino acids after amino acid 62 (insertion does not result in truncation). Insertion in first SH2 domain. The new variant retains all important sites including: the PROTEIN KINASE DOMAIN with two ATP BINDING sites, an ACTIVE SITE and an AUTO PHOSPHORYLATION site. An additional PHOSPHORYLATION site.

OSTEOPONTIN

OSTP_HUMAN

FUNCTION: BINDS TIGHTLY TO HYDROXYAPATITE. APPEARS TO FORM AN INTEGRAL PART OF THE MINERALIZED MATRIX. PROBABLY IMPORTANT TO CELL-MATRIX INTERACTION.
ALTERNATIVE PRODUCTS: TWO ISOFORMS; OP1A AND OP1B (SHOWN HERE); ARE PRODUCES BY ALTERNATIVE SPLICING.
PTM: EXTENSIVELY PHOSPHORYLATED ON SERINE RESIDUES.
PTM: N- AND O-GLYCOSYLATED.
DISEASE: THIS PROTEIN PLAYS A PRINCIPAL ROLE IN URINARY STONE FORMATION AS THE STONE MATRIX

NV_5

The new variant has an alternative 3' exon of 12 amino acids instead of 134 amino acids. The new variant maintains the CELL ATTACHMENT SITE and two GLYCOSILATION sites.

GADD45

GA45_HUMAN

GROWTH ARREST AND DNA-DAMAGE-INDUCIBLE PROTEIN
FUNCTION: INVOLVED IN THE REGULATION OF GROWTH AND APOPTOSIS. MEDIATES ACTIVATION OF STRESS-RESPONSIVE MTK1/MEKK4 MAPKKK.
SIMILARITY: BELONGS TO THE GADD45 / MYD118 FAMILY.

NV_6

The new variant has an alternative 5' exon of 72 amino acids instead of 125 amino acids. The new variant has a signal peptide and has the two PHOSPHORYLATION (BY CK2) sites.

WNT-11 PROTEIN

WN11_HUMAN

FUNCTION: PROBABLE DEVELOPMENTAL PROTEIN. MAY BE A SIGNALING MOLECULE WHICH AFFECT THE DEVELOPMENT OF DISCRETE REGIONS OF TISSUES. IS LIKELY TO SIGNAL OVER ONLY FEW CELL
DIAMETERS.
SUBCELLULAR LOCATION: POSSIBLY SECRETED AND ASSOCIATES WITH THE EXTRACELLULAR MATRIX.
SIMILARITY: BELONGS TO THE WNT FAMILY

NV_7

The new variant has a deletion of 22 amino acids after residue 312 (between 312-334). The new variant has all five potential GLYCOSILATION sites.

WNT-11 PROTEIN

WN11_HUMAN

NV_8

The new variant has a deletion of 117 amino acids after residue 116 (between 116-233). The new variant is missing one potential GLYCOSILATION site (out of 5 sites).

PROTEIN KINASE C, THETA TYPE

KPCT_HUMAN

FUNCTION: THIS IS CALCIUM-INDEPENDENT, PHOSPHOLIPID-DEPENDENT, SERINE- AND THREONINE-SPECIFIC ENZYME.
FUNCTION: PKC IS ACTIVATED BY DIACYLGLYCEROL WHICH IN TURN PHOSPHORYLATES A RANGE OF CELLULAR PROTEINS. PKC

ALSO SERVES AS THE RECEPTOR FOR PHORBOL ESTERS, A CLASS OF TUMOR
PROMOTERS.
TISSUE SPECIFICITY: SKELETAL MUSCLE, MEGAKARYOBLASTIC CELLS AND PLATELETS.
SIMILARITY: CONTAINS 2 ZINC-DEPENDENT PHORBOL-ESTER AND DAG BINDING DOMAINS.
SIMILARITY: BELONGS TO THE SER/THR FAMILY OF PROTEIN KINASES. PKC SUBFAMILY.

NV_9

The new variant has an alternative 3' exon of 3 amino acids instead of 94 amino acids. The alternative region is in the PROTEIN KINASE domain. The new variant maintains the two PHORBOL-ESTER AND DAG BINDING domains, the two ATP binding sites and the ACTIVE of the KINASE domain.

INTERFERON REGULATORY FACTOR 1

IRF1_HUMAN

FUNCTION: SPECIFICALLY BINDS TO THE UPSTREAM REGULATORY REGION OF TYPE I IFN AND IFN-INDUCIBLE MHC CLASS I GENES (THE INTERFERON CONSENSUS SEQUENCE (ICS)) AND ACTIVATES THOSE GENES.
SUBCELLULAR LOCATION: NUCLEAR.
INDUCTION: BY VIRUSES AND IFN.
DISEASE: DELETION OR REARRANGEMENT OF IRF1 ARE A CAUSE OF PRELEUKEMIC MYELODYSPLASTIC SYNDROME (MDS) AND OF ACUTE MYELOGENOUS LEUKEMIA (AML).
SIMILARITY: BELONGS TO THE IRF FAMILY.

NV_10

The new variant has an alternative 3' exon of 7 amino acids instead of 40 amino acids. The new variant maintains the DNA binding domain.

BASIC FIBROBLAST GROWTH FACTOR RECEPTOR 1

FGR1_HUMAN

FUNCTION: RECEPTOR FOR BASIC FIBROBLAST GROWTH FACTOR. A SHORTER FORM OF THE RECEPTOR COULD BE A RECEPTOR FOR ACIDIC FGF (AFGF).
CATALYTIC ACTIVITY: ATP + PROTEIN TYROSINE = ADP + PROTEIN TYROSINE PHOSPHATE.
SUBCELLULAR LOCATION: TYPE I MEMBRANE PROTEIN.
ALTERNATIVE PRODUCTS: MANY FORMS OF FGFR1 ARE PRODUCED BY ALTERNATIVE SPLICING. THE FORM SHOWN HERE IS KNOWN AS ALPHA-A1.
DISEASE: DEFECTS IN FGFR1 ARE ONE OF THE CAUSES OF PFEIFFER SYNDROME, ALSO CALLED ACROCEPHALOSYNDACTYLY TYPE V (ACS V), CHARACTERIZED BY CRANIOSYNOSTOSIS (PREMATURE FUSION OF THE SKULL SUTURES) WITH DEVIATION AND ENLARGEMENT OF THE THUMBS AND GREAT TOES,BRACHYMESOPHALANGY, WITH PHALANGEAL ANKYLOSIS AND A VARYING DEGREE OF SOFT TISSUE SYNDACTYLY.
SIMILARITY: BELONGS TO THE FIBROBLAST GROWTH FACTOR RECEPTOR FAMILY.
SIMILARITY: CONTAINS 3 IMMUNOGLOBULIN-LIKE DOMAINS.

NV_11

The new variant has an alternative 3' exon of 14 amino acids instead of 134 amino acids. The new variant has the entire extracellular domain and the TM, it is missing part of the cytoplasmic domain. The new variant maintains all 3 IMMUNOGLOBULIN-LIKE DOMAINS, the protein KINASE domain, the ACTIVE site, and the 2 ATP binding sites, but it might be missing one of the two PHOSPHORYLATION (AUTO-) sites.

REF-1 PROTEIN DNA-(APURINIC OR APYRIMIDINIC SITE) LYASE APE1_HUMAN

FUNCTION: REPAIRS OXIDATIVE DNA DAMAGES IN VITRO. MAY HAVE A ROLE IN PROTECTION AGAINST CELL LETHALITY AND SUPPRESSION OF MUTATIONS. REMOVES THE BLOCKING GROUPS FROM THE 3'

TERMINI OF THE DNA STRAND BREAKS GENERATED BY IONIZING RADIATIONS AND BLEOMYCIN.

CATALYTIC ACTIVITY: ENDONUCLEOLYTIC CLEAVAGE NEAR APURINIC OR APYRIMIDINIC SITES TO PRODUCTS WITH 5'-PHOSPHATE.

SUBCELLULAR LOCATION: NUCLEAR.

SIMILARITY: BELONGS TO THE AP/EXOA FAMILY OF DNA REPAIR ENZYMES.

NV_12

The new variant has a gap of 22 amino acids between residues 146 – 169. The new variant maintains the ACTIVE site and site important for substrate recognition.

NV_13

The new variant has an insertion of 25 amino acids after residue 18.It maintains the ACTIVE site and the site important for substrate recognition.

MAD3 MAJOR HISTOCOMPATIBILITY COMPLEX ENHANCER-BINDING PROTEIN MAD3_HUMAN

FUNCTION: I-KAPPA-B-LIKE ACTIVITY. MAY BE INVOLVED IN REGULATION OF TRANSCRIPTIONAL RESPONSES TO NF-KAPPA-B,

INCLUDING ADHESION- DEPENDENT PATHWAYS OF MONOCYTE ACTIVATION. INTERACTS DIRECTLY WITH THE NF-KAPPA-B COMPLEX, PRESUMABLY THROUGH THE P65 SUBUNIT.
INDUCTION: INDUCED IN ADHERENT MONOCYTES.
PTM: PHOSPHORYLATION OF I-KAPPA-B BLOCKS ITS ABILITY TO INHIBIT NF-KAPPA-B DNA-BINDING ACTIVITY.
SIMILARITY: CONTAINS 5 ANK REPEATS.

NV_14

The new variant has an alternative 3' exon of 3 amino acids instead of 15 amino acids. It retains all five ANK motifs and the two PHOSPHORYLATION sites.

NV_15

The new variant has a deletion of 28 amino acids between 183 – 212. The deletion is in the ANK MOTIF 4.The new variant maintains 4 out of the five ANK MOTIFs and the two PHORYLATION sites.

RECEPTOR PROTEIN-TYROSINE KINASE HEK8

EPA4_HUMAN

FUNCTION: RECEPTOR FOR MEMBERS OF THE EPHRIN-A FAMILY. BINDS TO EPHRIN-A1, -A4 AND -A5. BINDS MORE POORLY TO EPHRIN-A2 AND A-3.
CATALYTIC ACTIVITY: ATP + A PROTEIN TYROSINE = ADP + PROTEIN TYROSINE PHOSPHATE.
SUBCELLULAR LOCATION: TYPE I MEMBRANE PROTEIN.
SIMILARITY: CONTAINS 2 FIBRONECTIN TYPE III-LIKE DOMAINS.
SIMILARITY: TO OTHER PROTEIN-TYROSINE KINASES IN THE CATALYTIC DOMAIN. BELONGS TO THE EPHRIN RECEPTOR FAMILY.

NV_16

Deletion of 65 amino acids between 832 – 898. The deletion in the cytoplasmic domain. The 3' end of the PROTEIN KINASE domain is missing, but all important sites are maintained. The new variant has two FYBRONECTIN TYPE III domains and the protein KINASE domain with 2 ATP binding sites, an ACTIVE site and an auto PHOSPHORYLATION site.

C-ETS-2 PROTEIN
ETS2_HUMAN

SUBCELLULAR LOCATION: NUCLEAR.
SIMILARITY: BELONGS TO THE ETS FAMILY.

NV_17

The new variant has a deletion of 26 amino acids between 87 – 114. The new variant maintains the DNA binding domain.

WNT-5A PROTEIN
WN5A_HUMAN

FUNCTION: PROBABLE DEVELOPMENTAL PROTEIN. MAY BE A SIGNALING MOLECULE WHICH AFFECTS THE DEVELOPMENT OF DISCRETE REGIONS OF TISSUES. IS LIKELY TO SIGNAL OVER ONLY FEW CELL DIAMETERS.
SUBCELLULAR LOCATION: POSSIBLY SECRETED AND ASSOCIATES WITH THE EXTRACELLULAR MATRIX.
SIMILARITY: BELONGS TO THE WNT FAMILY

NV_18

The new variant has an alternative 3' exon of 4 amino acids instead of 109. It is identical to the known protein until residue 256. Two GLYCOSILATION sites out of four are missing in the new variant.

TYROSINE-PROTEIN KINASE SKY
TYO3_HUMAN

FUNCTION: MAY BE INVOLVED IN CELL ADHESION PROCESSES, PARTICULARLY IN THE CENTRAL NERVOUS SYSTEM.
SUBCELLULAR LOCATION: TYPE I MEMBRANE PROTEIN.
TISSUE SPECIFICITY: ABUNDANT IN THE BRAIN AND LOWER LEVELS IN OTHER TISSUES.
SIMILARITY: TO OTHER PROTEIN-TYROSINE KINASES IN THE CATALYTIC DOMAIN.
SIMILARITY: CONTAINS 2 IMMUNOGLOBULIN-LIKE C2-TYPE DOMAINS.
SIMILARITY: CONTAINS 2 FIBRONECTIN TYPE III-LIKE DOMAINS.

NV_19

The new variant has an alternative 3' exon of 45 amino acids instead of 216 amino acids. The new variant is missing part of the PROTEIN KINASE domain and its AUTOPHOSPHORYLATION site. However, it maintains all other necessary domains: the ACTIVE site and the two ATP binding sites. The variant retains all 6 GLYCOSILATION sites, the 2 IG-like domains and the 2 FIBRONEXTIN TYPE III domains.

NEURAL-CADHERIN
CAD2_HUMAN

FUNCTION: CADHERINS ARE CALCIUM DEPENDENT CELL ADHESION PROTEINS. THEY PREFERENTIALLY INTERACT WITH THEMSELVES

IN A HOMOPHILIC MANNER IN CONNECTING CELLS; CADHERINS MAY THUS CONTRIBUTE TO THE SORTING OF HETEROGENEOUS CELL TYPES. N-CADHERIN MAY BE INVOLVED IN NEURONAL RECOGNITION MECHANISM.
SUBCELLULAR LOCATION: TYPE I MEMBRANE PROTEIN.
SIMILARITY: BELONGS TO THE CADHERIN FAMILY.

NV_20

The new variant has an alternative 3' exon of 10 amino acids instead of 68 amino acids. The new variant maintains the extracellular domain and the TM domain. It is missing the end of the cytoplasmic domain and the SER-RICH domain. However, it has all other necessary domains including: 5 CADHERIN REPEATS with 7 GLYCOSILATION sites.

MXI1 MAX INTERACTING PROTEIN 1

MXI1_HUMAN

FUNCTION: TRANSCRIPTIONAL REPRESSOR. MXI1 BINDS WITH MAX TO FORM A SEQUENCE-SPECIFIC DNA-BINDING PROTEIN COMPLEX WHICH RECOGNIZES THE CORE SEQUENCE 5'-CAC[GA]TG-3'. MXI1 THUS ANTAGONIZES MYC TRANSCRIPTIONAL ACTIVITY BY COMPETING FOR MAX.
SUBUNIT: EFFICIENT DNA BINDING REQUIRES DIMERIZATION WITH ANOTHER BHLH PROTEIN.BINDS DNA AS A HETERODIMER WITH MAX.
SUBCELLULAR LOCATION: NUCLEAR.
TISSUE SPECIFICITY: HIGH LEVELS FOUND IN THE BRAIN, HEART AND LUNG WHILE LOWER LEVELS ARE SEEN IN THE LIVER, KIDNEY AND SKELETAL MUSCLE.
DISEASE: DEFECTS IN MXI1 ARE FOUND IN SOME PATIENTS WITH PROSTATE TUMORS.
SIMILARITY: BELONGS TO THE BASIC HELIX-LOOP-HELIX (BHLH) FAMILY OF TRANSCRIPTION FACTORS.

NV_21

The new variant has an insertion of 24 amino acids after residue 79. It is most likely truncated within the insertion. The new variant retains the BASIC DNA BINDING domain, but lacks the HELIX LOOP HELIX motif.

MXI1 MAX INTERACTING PROTEIN 1

MXI1_HUMAN

NV_22

The new variant has an alternative 5' exon of 31 amino acids instead of 25. It is identical to the known protein from residue 26 to the end. The new variant has both the DNA BINDING DOMAIN and the HELIX LOOP HELIX motif. The alternative 5' exon bares a clathrin repeat.

DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE 3

MPK3_HUMAN

FUNCTION: DUAL SPECIFICITY KINASE. IS ACTIVATED BY CYTOKINES AND ENVIRONMENTAL STRESS IN VIVO. CATALYZES THE CONCOMITANT PHOSPHORYLATION OF A THREONINE AND A TYROSINE RESIDUE IN THE MAP KINASE P38.
ENZYME REGULATION: ACTIVATED BY DUAL PHOSPHORYLATION ON SER-189 AND THR-193.
TISSUE SPECIFICITY: ABUNDANT EXPRESSION IS SEEN IN THE SKELETAL MUSCLE. IT IS ALSO WIDELY EXPRESSED IN OTHER TISSUES.
PTM: AUTOPHOSPHORYLATED.
SIMILARITY: BELONGS TO THE SER/THR FAMILY OF PROTEIN KINASES. MAP KINASE KINASE SUBFAMILY.

NV_23

The new variant has an alternative 3' exon of 10 amino acids instead of 28 amino acids. The new variant maintains the PROTEIN KINASE domain with its two ATP binding sites, the ACTIVE site and two POSPHORYLATION sites.It may lack a few amino acids at the end of the PROTEIN KINASE domain.

DNA-REPAIR PROTEIN XRCC1
XRC1_HUMAN

FUNCTION: CORRECTS DEFECTIVE DNA STRAND-BREAK REPAIR AND SISTER CHROMATID EXCHANGE FOLLOWING TREATMENT WITH IONIZING RADIATION AND ALKYLATING AGENTS.
SUBCELLULAR LOCATION: NUCLEAR (PROBABLE).
SIMILARITY: SOME, TO S.POMBE RAD4/CUT5.

NV_24

Alternative 3' exon of 50 amino acids instead of 391 amino acids.

DNA-REPAIR PROTEIN XRCC1
XRC1_HUMAN

NV_25

Alternative 3' exon of 25 amino acids instead of 392 amino acids.

DNA-REPAIR PROTEIN XRCC1
XRC1_HUMAN

NV_26

Alternative 3' exon of 61 amino acids instead of 447 amino acids.

DNA-REPAIR PROTEIN XRCC1
XRC1_HUMAN

NV_27

Alternative 3' exon of 84 amino acids instead of 93 amino acids.

MERLIN SCHWANNOMIN (NF2)
MERL_HUMAN

FUNCTION: PROBABLY ACTS AS A MEMBRANE STABILIZING PROTEIN.
TISSUE SPECIFICITY: IN FETAL BRAIN; IN KIDNEY, LUNG, BREAST, AND OVARY.
DISEASE: NEUROFIBROMATOSIS 2 (NF2) OR CENTRAL NEUROFIBROMATOSIS IS A GENETIC DISORDER CHARACTERIZED BY BILATERAL VESTIBULAR SCHWANNOMAS (FORMERLY CALLED ACOUSTIC NEUROMAS), SCHWANNOMAS OF OTHER CRANIAL AND PERIPHERAL NERVES,MENINGIOMAS, AND EPENDYMOMAS. IT IS INHERITED IN AN AUTOSOMAL DOMINANT FASHION WITH FULL PENETRANCE. AFFECTED INDIVIDUALS GENERALLY DEVELOP SYMPTOMS
OF EIGHTH-NERVE DYSFUNCTION IN EARLY ADULTHOOD, INCLUDING DEAFNESS AND BALANCE DISORDER. ALTHOUGH THE TUMORS OF NF2 ARE HISTOLOGICALLY BENIGN, THEIR ANATOMIC LOCATION MAKES MANAGEMENT DIFFICULT, AND PATIENTS SUFFER GREAT MORBIDITY AND MORTALITY.
SIMILARITY: CONTAINS A DOMAIN FOUND IN BAND 4.1, EZRIN, MOESIN, RADIXIN, AND TALIN.

NV_28

The new variant has a deletion of 29 amino acids after residue 333.The new variant maintains the BAND 4,1 – LIKE domain. (Band 4.1, which links the spectrin-actin cytoskeleton of erythrocytes to the plasma membrane).

DP1 POLYPOSIS LOCUS PROTEIN 1
DP1_HUMAN

SUBCELLULAR LOCATION: INTEGRAL MEMBRANE PROTEIN (POTENTIAL).
SIMILARITY: TO C.ELEGANS T19C3.4.

NV_29

Alternative 3' exon of 21 amino acids instead of 72 amino acids. The new variant maintains the two transmembrane domains.

MDR1 MULTIDRUG RESISTANCE PROTEIN 1
MDR1_HUMAN

FUNCTION: ENERGY-DEPENDENT EFFLUX PUMP RESPONSIBLE FOR DECREASED DRUG ACCUMULATION IN MULTIDRUG-RESISTANT CELLS.
SUBCELLULAR LOCATION: INTEGRAL MEMBRANE PROTEIN.
SIMILARITY: BELONGS TO THE ATP-BINDING TRANSPORT PROTEIN FAMILY (ABC TRANSPORTERS). MDR SUBFAMILY.

NV_30

The new variant has an alternative 3' exon of 1 amino acid, instead of 3 of the known protein. The new variant is identical to the known protein until residue 1277. It maintains all important sites.

MDR1 MULTIDRUG RESISTANCE PROTEIN 1
MDR1_HUMAN

NV_31

The new variant is a truncated protein. It has an alternative 3' exon of 12 amino acids instead of 713. It is identical to the known protein until residue 567.

The new variant retains only one out of two ATP binding sites, and six out of twelve TM domains. It has one out of three cytoplasmic domains and is truncated in the middle of the second cytoplasmic domain.

JNK1 – MITOGEN ACTIVATED PROTEIN KINASE 8
MK08_HUMAN

FUNCTION: PROBABLY PLAYS A ROLE IN THE SIGNAL TRANSDUCTION PATHWAY INITIATED BY PROINFLAMMATORY CYTOKINES AND UV RADIATION. BINDS TO THE N-TERMINAL ACTIVATION DOMAINS OF C-JUN AND ATF2 AND PHOSPHORYLATES THEIR REGULATORY SITES (RESPECTIVELY SER-63 AND SER-73; THR-69 AND THR-71). JNK1 ISOFORMS DISPLAY DIFFERENT BINDING PATTERNS: BETA-1 PREFERENTIALLY BINDS TO C-JUN, WHEREAS ALPHA-1, ALPHA-2, AND BETA-2 HAVE A SIMILAR LOW LEVEL OF BINDING TO BOTH C-JUN OR ATF2. HOWEVER THERE IS NO CORRELATION BETWEEN BINDING AND PHOSPHORYLATION, WHICH IS ACHIEVED ABOUT AT THE SAME EFFICIENCY BY ALL ISOFORMS.
ENZYME REGULATION: ACTIVATED BY THREONINE AND TYROSINE PHOSPHORYLATION.
ALTERNATIVE PRODUCTS: FOUR ISOFORMS JNK1 ALPHA-1, JNK1 ALPHA-2 (SHOWN HERE), JNK1 BETA-1, AND JNK1 BETA-2 ARE PRODUCED BY ALTERNATIVE SPLICING.
INDUCTION: BY UV LIGHT, INTERLEUKIN-1 AND BY HA-RAS.
SIMILARITY: BELONGS TO THE CDC2/CDC28 SUBFAMILY OF SER/THR PROTEIN KINASES. STRONGEST SIMILARITY WITH OTHER MAP KINASES.

NV_32

The new variant is a truncated protein. It has an alternative 3' exon of 13 amino acids instead of 222 amino acids. It is identical to the known protein until residue 205. The new variant lacks part of the PROTEIN KINASE domain, however it retains the ACTIVE SITE, the two ATP binding sites and the two PHOSPHORYLATION sites.

JNK1 – MITOGEN ACTIVATED PROTEIN KINASE 8
MK08_HUMAN

NV_33

The new variant has an alternative 3' exon of 14 amino acids instead of 134 amino acids of the known protein. It is identical to the known protein until residue 293. The new variant lacks the end of the PROTEIN KINASE domain, but retains the ACTIVE SITE, the two ATP binding sites and the two PHOSPHORYLATION sites.

JNK1 – MITOGEN ACTIVATED PROTEIN KINASE 8
MK08_HUMAN

NV_34

The new variant has an alternative 3' exon of 7 amino acids a instead of 95 amino acids. It is identical to the known protein until residue 332. Has the entire PROTEIN KINASE domain including the ACTIVE SITE, the two ATP binding sites and the two PHOSPHORYLATION sites.

MITOGEN-ACTIVATED PROTEIN KINASE 12

Synonym(s) EC 2.7.1- extracellular signal-regulated kinase 6 EC 2.7.1-ERK 6 ERK 5 stress-activated protein kinase-3 mitogen-activated protein kinase P38 gamma map kinase P38 gamma

Gene name(s) MAPK12 or ERK 6 or SAPK 3

FUNCTION: PHOSPHORYLATES MYELIN BASIC PROTEIN (MBP); ACTS AS SIGNAL TRANSDUCER DURING THE DIFFERENTIATION OF MYOBLASTS TO MYOTUBES. OVEREXPRESSION ENHANCES THIS

DIFFERENTIATION EVENT, WHEREAS INACTIVATION EXHIBITS IT AND MAINTAINS THE CELLS IN A PROLIFERATIVE STATE.

ENZYME REGULATION: ACTIVATED BY PHSOPHORYLATION ON THREONINE AND TYROSINE (BY SIMILARITY).

TISSUE SPECIFICITY: HIGHLY EXPRESSED IN SKELETAL MUSCLE.

SIMILARITY: BELONGS TO THE SER/THR FAMILY OF PROTEIN KINASES. MAP KINASE FAMILY.

NV_35

The new variant contains 152 N-terminal amino acids of the original protein. The new variant has alternative 25 amino acids in its C-terminus, instead of original 215 amino acids. It contains the NP_BIND (between the amino acids 33 – 41, and the ATP binding site at position 56. The truncated variant has only part of the kinase domain, it lacks the active site and both the phosphorylation sites that activates the kinase. This truncated splice variant can act as dominant negative.

PROTEIN KINASE C, THETA TYPE
KPCT_HUMAN

FUNCTION: THIS IS CALCIUM-INDEPENDENT, PHOSPHOLIPID-DEPENDENT, SERINE- AND THREONINE-SPECIFIC ENZYME.

FUNCTION: PKC IS ACTIVATED BY DIACYLGLYCEROL WHICH IN TURN PHOSPHORYLATES A RANGE OF CELLULAR PROTEINS. PKC ALSO SERVES AS THE RECEPTOR FOR PHORBOL ESTERS, A CLASS OF TUMOR PROMOTERS.

TISSUE SPECIFICITY: SKELETAL MUSCLE, MEGAKARYOBLASTIC CELLS AND PLATELETS.

SIMILARITY: CONTAINS 2 ZINC-DEPENDENT PHORBOL-ESTER AND DAG BINDING DOMAINS.

SIMILARITY: BELONGS TO THE SER/THR FAMILY OF PROTEIN KINASES. PCK SUBFAMILY.

NV_36

The new variant has an alternative 3′ exon of 36 amino acids instead of 94 original amino acids. The alternative region is in the PROTEIN KINASE domain. The new variant maintains the two PHORBOL-ESTER AND DAG BINDING domains, the two ATP binding sites and the ACTIVE of the KINASE domain.

Example II: Variant nucleic acid sequence

The nucleic acid sequences of the invention include nucleic acid sequences which encode variant product and fragments and analogs thereof. The nucleic acid sequences may alternatively be sequences complementary to the above coding sequence, or to a region of said coding sequence. The length of the complementary sequence is sufficient to avoid the expression of the coding sequence. The nucleic acid sequences may be in the form of RNA or in the form of DNA, and include messenger RNA, synthetic RNA and DNA, cDNA, and genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded may be the coding strand or the non-coding (anti-sense, complementary) strand. The nucleic acid sequences may also both include dNTPs, rNTPs as well as non naturally occurring sequences. The sequence may also be a part of a hybrid between an amino acid sequence and a nucleic acid sequence.

In a general embodiment, the nucleic acid sequence has at least 90%, identity with any one of the sequence identified as SEQ ID NO: 1 to SEQ ID NO: 36 provided that this sequence is not completely identical with that of the original sequence.

The nucleic acid sequences may include the coding sequence by itself. By another alternative the coding region may be in combination with additional coding sequences, such as those coding for fusion protein or signal peptides, in

combination with non-coding sequences, such as introns and control elements, promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or in a vector or host environment in which the variant nucleic acid sequence is introduced as a heterologous sequence.

The nucleic acid sequences of the present invention may also have the product coding sequence fused in-frame to a marker sequence which allows for purification of the variant product. The marker sequence may be, for example, a hexahistidine tag to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al. Cell* **37**:767 (1984)).

Also included in the scope of the invention are fragments as defined above also referred to herein as oligonucleotides, typically having at least 20 bases, preferably 20-30 bases corresponding to a region of the coding-sequence nucleic acid sequence. The fragments may be used as probes, primers, and when complementary also as antisense agents, and the like, according to known methods.

As indicated above, the nucleic acid sequence may be substantially a depicted in any one of SEQ ID NO: 1 to SEQ ID NO: 36 or fragments thereof or sequences having at least 90% identity to the above sequence as explained above. Alternatively, due to the degenerative nature of the genetic code, the sequence may be a sequence coding for any one of the amino acid sequence of SEQ ID NO: 37 to SEQ ID NO: 72, or fragments or analogs of said amino acid sequence.

A. Preparation of nucleic acid sequences

The nucleic acid sequences may be obtained by screening cDNA libraries using oligonucleotide probes which can hybridize to or PCR-amplify nucleic acid sequences which encode the variant products disclosed above. cDNA libraries

prepared from a variety of tissues are commercially available and procedures for screening and isolating cDNA clones are well-known to those of skill in the art. Such techniques are described in, for example, Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (2nd Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

The nucleic acid sequences may be extended to obtain upstream and downstream sequences such as promoters, regulatory elements, and 5' and 3' untranslated regions (UTRs). Extension of the available transcript sequence may be performed by numerous methods known to those of skill in the art, such as PCR or primer extension (Sambrook *et al., supra*), or by the RACE method using, for example, the Marathon RACE kit (Clontech, Cat. # K1802-1).

Alternatively, the technique of *"restriction-site"* PCR (Gobinda *et al. PCR Methods Applic.* **2**:318-22, (1993)), which uses universal primers to retrieve flanking sequence adjacent a known locus, may be employed. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. *et al.*, *Nucleic Acids Res.* **16**:8186, (1988)). The primers may be designed using OLIGO(R) 4.06 Primer Analysis Software (1992; National Biosciences Inc, Plymouth, Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom, M. *et al., PCR Methods Applic.* **1**:111-19, (1991)) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into a flanking part of the DNA molecule before PCR.

Another method which may be used to retrieve flanking sequences is that of Parker, J.D., *et al., Nucleic Acids Res.*, **19**:3055-60, (1991)). Additionally, one can use PCR, nested primers and PromoterFinder™ libraries to *"walk in"* genomic DNA (PromoterFinder™; Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. Preferred libraries for screening for full length cDNAs are ones that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes.

A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for extension into the 5' nontranslated regulatory region.

The nucleic acid sequences and oligonucleotides of the invention can also be prepared by solid-phase methods, according to known synthetic methods. Typically, fragments of up to about 100 bases are individually synthesized, then joined to form continuous sequences up to several hundred bases.

B. Use of variant nucleic acid sequence for the production of variant products

In accordance with the present invention, nucleic acid sequences specified above may be used as recombinant DNA molecules that direct the expression of variant products.

As will be understood by those of skill in the art, it may be advantageous to produce variant product-encoding nucleotide sequences possessing codons

other than those which appear in any one of SEQ ID NO: 1 to SEQ ID NO: 36 which are those which naturally occur in the human genome. Codons preferred by a particular prokaryotic or eukaryotic host (Murray, E. *et al. Nuc Acids Res.*, **17**:477-508, (1989)) can be selected, for example, to increase the rate of variant product expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

The nucleic acid sequences of the present invention can be engineered in order to alter a variant product coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.

The present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are also described in Sambrook, *et al., (supra)*.

The present invention also relates to host cells which are genetically engineered with vectors of the invention, and the production of the product of the invention by recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The

engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the expression of the variant nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art.

The nucleic acid sequences of the present invention may be included in any one of a variety of expression vectors for expressing a product. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host. The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and related sub-cloning procedures are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate transcription control sequence (promoter) to direct mRNA synthesis. Examples of such promoters include: LTR or SV40 promoter, the *E.coli lac* or *trp* promoter, the phage lambda *PL* promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation, and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E.coli.*

The vector containing the appropriate DNA sequence as described above, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. Examples of appropriate expression hosts include: bacterial cells, such as *E.coli*, *Streptomyces, Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* and *Spodoptera* Sf9; animal cells such as CHO, COS, HEK 293 or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein. The invention is not limited by the host cells employed.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the variant product. For example, when large quantities of variant product are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, multifunctional *E.coli* cloning and expression vectors such as *Bluescript*(R) (Stratagene), in which the variant polypeptide coding sequence may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta-galactosidase so that a hybrid protein is produced; *pIN* vectors (Van Heeke & Schuster *J. Biol. Chem.* **264**:5503-5509, (1989)); *pET* vectors (Novagen, Madison WI); and the like.

In the yeast *Saccharomyces cerevisiae* a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For reviews, see Ausubel *et al. (supra)* and Grant *et al.*, (*Methods in Enzymology* **153**:516-544, (1987)).

In cases where plant expression vectors are used, the expression of a sequence encoding variant product may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of *CaMV* (Brisson *et al., Nature* **310**:511-514. (1984)) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu *et al.*, *EMBO J.*, **6**:307-311, (1987)). Alternatively, plant promoters such as the small

subunit of RUBISCO (Coruzzi *et al.*, *EMBO J.* **3**:1671-1680, (1984); Broglie *et al.*, *Science* **224**:838-843, (1984)); or heat shock promoters (Winter J and Sinibaldi R.M., *Results Probl. Cell Differ.,* **17**:85-105, (1991)) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S. or Murry L.E. (1992) in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y., pp 191-196; or Weissbach and Weissbach (1988) *Methods for Plant Molecular Biology*, Academic Press, New York, N.Y., pp 421-463.

Variant product may also be expressed in an insect system. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The variant product coding sequence may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of variant coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which variant protein is expressed (Smith *et al., J. Virol.* 46:584, (1983); Engelhard, E.K. *et al., Proc. Nat. Acad. Sci.* **91**:3224-7, (1994)).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a variant product coding sequence may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing variant protein in infected host cells (Logan and Shenk, *Proc. Natl. Acad. Sci.* **81**:3655-59, (1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of a variant product coding sequence. These signals include the ATG initiation

codon and adjacent sequences. In cases where variant product coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf, D. *et al.,* (1994) *Results Probl. Cell Differ.,* **20**:125-62, (1994); Bittner *et al., Methods in Enzymol* **153**:516-544, (1987)).

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., and Battey, I. (1986) Basic Methods in Molecular Biology). Cell-free translation systems can also be employed to produce polypeptides using RNAs derived from the DNA constructs of the present invention.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a *"pre-pro"* form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational

activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express variant product may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M., *et al.*, *Cell* **11**:223-32, (1977)) and adenine phosphoribosyltransferase (Lowy I., *et al.*, *Cell* **22**:817-23, (1980)) genes which can be employed in *tk-* or *aprt-* cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, *dhfr* which confers resistance to methotrexate (Wigler M., *et al., Proc. Natl. Acad. Sci.* **77**:3567-70, (1980)); *npt*, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al., J. Mol. Biol.*, **150**:1-14, (1981)) and *als* or *pat*, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman S.C. and R.C. Mulligan, *Proc. Natl. Acad. Sci.* **85**:8047-51, (1988)). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate, GUS, and luciferase and its substrates, luciferin and ATP, being widely used not only to identify transformants, but also to quantify the amount of transient or stable

protein expression attributable to a specific vector system (Rhodes, C.A. *et. al., Methods Mol. Biol.,* 55:121-131, (1995)).

Host cells transformed with a nucleotide sequence encoding variant product may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The product produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing nucleic acid sequences encoding variant product can be designed with signal sequences which direct secretion of variant product through a prokaryotic or eukaryotic cell membrane.

The variant product may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, Wash.). The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and variant product is useful to facilitate purification. One such expression vector provides for expression of a fusion protein compromising a variant polypeptide fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography, as described in Porath, *et al., Protein Expression and Purification,* **3**:263-281, (1992)) while the enterokinase cleavage site provides a means for isolating variant polypeptide from the fusion protein. *pGEX* vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to ligand-agarose beads (e.g., glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art.

The variant products can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

C. Diagnostic applications utilizing nucleic acid sequences

The nucleic acid sequences of the present invention may be used for a variety of diagnostic purposes. The nucleic acid sequences may be used to detect and quantitate expression of the variant in patient's cells, e.g. biopsied tissues, by detecting the presence of mRNA coding for variant product. Alternatively, the assay may be used to detect soluble variant in the serum or blood. This assay typically involves obtaining total mRNA from the tissue or serum and contacting the mRNA with a nucleic acid probe. The probe is a nucleic acid molecule of at least 20 nucleotides, preferably 20-30 nucleotides, capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding variant product under hybridizing conditions, detecting the

presence of mRNA hybridized to the probe, and thereby detecting the expression of variant. This assay can be used to distinguish between absence, presence, and excess expression of variant product and to monitor levels of variant expression during therapeutic intervention. In addition, the assay may be used to compare the levels of the variant of the invention to the levels of the original sequence from which it has been varied or to levels of other variants, which comparison may have some physiological meaning.

The invention also contemplates the use of the nucleic acid sequences as a diagnostic for diseases resulting from inherited defective variant sequences, or diseases in which the ratio of the amount of the original sequence from which the variant was varied to the novel variants of the invention is altered. These sequences can be detected by comparing the sequences of the defective (i.e., mutant) variant coding region with that of a normal coding region. Association of the sequence coding for mutant variant product with abnormal variant product activity may be verified. In addition, sequences encoding mutant variant products can be inserted into a suitable vector for expression in a functional assay system (e.g., colorimetric assay, complementation experiments in a variant protein deficient strain of HEK293 cells) as yet another means to verify or identify mutations. Once mutant genes have been identified, one can then screen populations of interest for carriers of the mutant gene.

Individuals carrying mutations in the nucleic acid sequence of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis may be obtained from a patient's cells, including but not limited to such as from blood, urine, saliva, placenta, tissue biopsy and autopsy material. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki, *et al.*, *Nature* **324**:163-166, (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid of the present invention can be used to identify and analyze mutations in the gene of the present invention.

Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype.

Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA of the invention or alternatively, radiolabeled antisense DNA sequences of the invention. Sequence changes at specific locations may also be revealed by nuclease protection assays, such RNase and S1 protection or the chemical cleavage method (e.g. Cotton, *et alProc. Natl. Acad. Sci.* USA, **85**:4397-4401, (1985)), or by differences in melting temperatures. *"Molecular beacons"* (Kostrikis L.G. *et al.*, Science **279**:1228-1229, (1998)), hairpin-shaped, single-stranded synthetic oligonucleotides containing probe sequences which are complementary to the nucleic acid of the present invention, may also be used to detect point mutations or other sequence changes as well as monitor expression levels of variant product. Such diagnostics would be particularly useful for prenatal testing.

Another method for detecting mutations uses two DNA probes which are designed to hybridize to adjacent regions of a target, with abutting bases, where the region of known or suspected mutation(s) is at or near the abutting bases. The two probes may be joined at the abutting bases, e.g., in the presence of a ligase enzyme, but only if both probes are correctly base paired in the region of probe junction. The presence or absence of mutations is then detectable by the presence or absence of ligated probe.

Also suitable for detecting mutations in the variant product coding sequence are oligonucleotide array methods based on sequencing by hybridization (SBH), as described, for example, in U.S. Patent No. 5,547,839. In a typical method, the DNA target analyte is hybridized with an array of oligonucleotides formed on a microchip. The sequence of the target can then be "read" from the pattern of target binding to the array.

D. Gene mapping utilizing nucleic acid sequences

The nucleic acid sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 20-30 bp) from the variant cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, which would complicate the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids or using instead radiation hybrids are rapid procedures for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bases. For a review of this technique, see Verma *et al., Human Chromosomes: a Manual of Basic Techniques*, (1988) Pergamon Press, New York.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in the OMIM database (Center for Medical Genetics, Johns Hopkins University, Baltimore, MD and National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD). The OMIM gene map presents the cytogenetic map location of disease genes and other expressed genes. The OMIM database provides information on diseases associated with the chromosomal location. Such associations include the results of linkage analysis mapped to this interval, and the correlation of translocations and other chromosomal aberrations in this area with the advent of polygenic diseases, such as cancer, in general and prostate cancer in particular.

E. Therapeutic applications of nucleic acid sequences

Nucleic acid sequences of the invention may also be used for therapeutic purposes. Turning first to the second aspect of the invention (i.e. inhibition of expression of variant), expression of variant product may be modulated through antisense technology, which controls gene expression through hybridization of complementary nucleic acid sequences, i.e. antisense DNA or RNA, to the control, 5' or regulatory regions of the gene encoding variant product. For example, the 5' coding portion of the nucleic acid sequence sequence which codes for the product of the present invention is used to design an antisense oligonucleotide of from about 10 to 40 base pairs in length. Oligonucleotides derived from the transcription start site, e.g. between positions -10 and +10 from the start site, are preferred. An antisense DNA oligonucleotide is designed to be complementary to a region of the nucleic acid sequence involved in transcription (Lee *et al., Nucl. Acids, Res*., **6**:3073, (1979); Cooney et al., Science **241**:456, (1988); and Dervan *et al., Science* **251**:1360, (1991)), thereby preventing transcription and the production of the variant products. An antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the

mRNA molecule into the variant products (Okano *J. Neurochem.* **56**:560, (1991)). The antisense constructs can be delivered to cells by procedures known in the art such that the antisense RNA or DNA may be expressed *in vivo*. The antisense may be antisense mRNA or DNA sequence capable of coding such antisense mRNA. The antisense mRNA or the DNA coding thereof can be complementary to the full sequence of nucleic acid sequences coding for the variant protein or to a fragment of such a sequence which is sufficient to inhibit production of a protein product.

Turning now to the first aspect of the invention, i.e. expression of variant, expression of variant product may be increased by providing coding sequences for coding for said product under the control of suitable control elements ending its expression in the desired host.

The nucleic acid sequences of the invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The products of the invention as well as any activators and deactivators compounds (see below) which are polypeptides, may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as *"gene therapy."* Cells from a patient may be engineered with a nucleic acid sequence (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered in vivo for expression of a polypeptide *in vivo* by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the

present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a product of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors mentioned above may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the *PE501*, *PA317*, *psi-2*, *psi-AM*, *PA12*, *T19-14X*, *VT-19-17-H2*, *psi-CRE*, *psi-CRIP*, *GP+E-86*, *GP+envAm12*, and *DAN* cell lines as described in Miller (*Human Gene Therapy*, Vol. **1**, pg. 5-14, (1990)). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The genes introduced into cells may be placed under the control of inducible promoters, such as the radiation-inducible Egr-1 promoter, (Maceri, H.J., *et al., Cancer Res.*, **56**(19):4311 (1996)), to stimulate variant production or antisense inhibition in response to radiation, eg., radiation therapy for treating tumors.

Example III. Variant product

The substantially purified variant product of the invention has been defined above as the product coded from the nucleic acid sequence of the invention. Preferably the amino acid sequence is an amino acid sequence having at least 90% identity to any one of the sequences identified as SEQ ID NO: 37 to SEQ ID NO: 72 provided that the amino acid sequence is not identical to that of the original sequence from which it has been varied. The protein or polypeptide may be in mature and/or modified form, also as defined above. Also contemplated are protein fragments having at least 10 contiguous amino acid residues, preferably at least 10-20 residues, derived from the variant product, as well as homologues as explained above.

The sequence variations are preferably those that are considered conserved substitutions, as defined above. Thus, for example, a protein with a sequence having at least 90% sequence identity with any of the products identified as SEQ ID NO: 37 to SEQ ID NO: 72, preferably by utilizing conserved substitutions as defined above is also part of the invention, and provided that it is not identical to the original peptide from which it has been varied. In a more specific embodiment, the protein has or contains any one of the sequence identified as SEQ ID NO: 37 to SEQ ID NO: 72. The variant product may be (i) one in which one or more of the amino acid residues in a sequence listed above are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the variant product is fused with another compound, such as a compound to increase the half-life of the

protein (for example, polyethylene glycol (PEG)), or a moiety which serves as targeting means to direct the protein to its target tissue or target cell population (such as an antibody), or (iv) one in which additional amino acids are fused to the variant product. Such fragments, variants and derivatives are deemed to be within the scope of those skilled in the art from the teachings herein.

A. Preparation of variant product

Recombinant methods for producing and isolating the variant product, and fragments of the protein are described above.

In addition to recombinant production, fragments and portions of variant product may be produced by direct peptide synthesis using solid-phase techniques (cf. Stewart *et al.*, (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J., *J. Am. Chem. Soc.,* **85**:2149-2154, (1963)). In vitro peptide synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.) in accordance with the instructions provided by the manufacturer. Fragments of variant product may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

II. Therapeutic uses and compositions utilizing the variant product

The variant product of the invention is generally useful in treating diseases and disorders which are characterized by a lower than normal level of variant expression, and or diseases which can be cured or ameliorated by raising the level of the variant product, even if the level is normal.

Variant products or fragments may be administered by any of a number of routes and methods designed to provide a consistent and predictable concentration of compound at the target organ or tissue. The product-containing compositions may be administered alone or in combination with other agents,

such as stabilizing compounds, and/or in combination with other pharmaceutical agents such as drugs or hormones.

Variant product-containing compositions may be administered by a number of routes including, but not limited to oral, intravenous, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means as well as by nasal application. Variant product-containing compositions may also be administered via liposomes. Such administration routes and appropriate formulations are generally known to those of skill in the art.

The product can be given via intravenous or intraperitoneal injection. Similarly, the product may be injected to other localized regions of the body. The product may also be administered via nasal insufflation. Enteral administration is also possible. For such administration, the product should be formulated into an appropriate capsule or elixir for oral administration, or into a suppository for rectal administration.

The foregoing exemplary administration modes will likely require that the product be formulated into an appropriate carrier, including ointments, gels, suppositories. Appropriate formulations are well known to persons skilled in the art.

Dosage of the product will vary, depending upon the potency and therapeutic index of the particular polypeptide selected.

A therapeutic composition for use in the treatment method can include the product in a sterile injectable solution, the polypeptide in an oral delivery vehicle, the product in an aerosol suitable for nasal administration, or the product in a nebulized form, all prepared according to well known methods. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The product of the invention may also be used to modulate endothelial differentiation and proliferation as well as to modulate apoptosis either *ex vivo* or *in vitro*, for example, in cell cultures.

Example IV. Screening methods for activators and deactivators (inhibitors)

The present invention also includes an assay for identifying molecules, such as synthetic drugs, antibodies, peptides, or other molecules, which have a modulating effect on the activity of the variant product, e.g. activators or deactivators of the variant product of the present invention. Such an assay comprises the steps of providing an variant product encoded by the nucleic acid sequences of the present invention, contacting the variant protein with one or more candidate molecules to determine the candidate molecules modulating effect on the activity of the variant product, and selecting from the molecules a candidate's molecule capable of modulating variant product physiological activity.

The variant product, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment employed in such a test may be free in solution, affixed to a solid support, borne on a cell membrane or located intracellularly. The formation of binding complexes, between variant product and the agent being tested, may be measured. Alternatively, the activator or deactivator may work by serving as agonist or antagonist, respectively, of the variant receptor, binding entity or target site, and their effect may be determined in connection with any of the above.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the variant product is described in detail by Geysen in PCT Application WO 84/03564, published on Sep. 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with the full variant product or with fragments of variant product and washed. Bound variant product is then detected by methods well known in the art. Substantially purified

variant product can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

Antibodies to the variant product, as described in Example VI below, may also be used in screening assays according to methods well known in the art. For example, a *"sandwich"* assay may be performed, in which an anti-variant antibody is affixed to a solid surface such as a microtiter plate and variant product is added. Such an assay can be used to capture compounds which bind to the variant product. Alternatively, such an assay may be used to measure the ability of compounds to influence with the binding of variant product to the variant receptor, and then select those compounds which effect the binding.

Example V. Anti-variant antibodies

A. Synthesis

In still another aspect of the invention, the purified variant product is used to produce anti-variant antibodies which have diagnostic and therapeutic uses related to the activity, distribution, and expression of the variant product.

Antibodies to the variant product may be generated by methods well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies, i.e., those which inhibit dimer formation, are especially preferred for therapeutic use.

A fragment of the variant product for antibody induction does not require biological activity but have to feature immunological activity; however, the protein fragment or oligopeptide must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids of the sequences specified in any one of SEQ ID NO: 37 to SEQ ID NO: 72. Preferably they should mimic a portion of the amino acid sequence of the natural protein and may contain the

entire amino acid sequence of a small, naturally occurring molecule. Short stretches of variant protein amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to variant product.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc may be immunized by injection with variant product or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants.

Monoclonal antibodies to variant protein may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (*Nature* **256**:495-497, (1975)), the human B-cell hybridoma technique (Kosbor *et al., Immunol. Today* **4**:72, (1983); Cote *et al., Proc. Natl. Acad. Sci.* **80**:2026-2030, (1983)) and the EBV-hybridoma technique (Cole, *et al., Mol. Cell Biol.* 62:109-120, (1984)).

Techniques developed for the production of *"chimeric antibodies"*, the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can also be used (Morrison *et al., Proc. Natl. Acad. Sci.* **81**:6851-6855, (1984); Neuberger *et al., Nature* **312**:604-608, (1984); Takeda *et al., Nature* **314**:452-454, (1985)). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single-chain antibodies specific for the variant protein.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al.* (*Proc. Natl. Acad. Sci.* **86**:3833-3837, 1989)), and Winter G and Milstein C., (*Nature* **349**:293-299, (1991)).

Antibody fragments which contain specific binding sites for variant protein may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse W.D. *et al., Science* 256:1275-1281, (1989)).

B. Diagnostic applications of antibodies

A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between the variant product and its specific antibody and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on a specific variant product is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox D.E., *et al.,* (*J. Exp. Med.* **158**:1211, (1983)).

Antibodies which specifically bind variant product are useful for the diagnosis of conditions or diseases characterized by expression of the novel variant of the invention (where normally it is not expressed) by over or under expression of variant as well as for detection of diseases in which the proportion between the amount of the variants of the invention and the original sequence from which it varied is altered. Alternatively, such antibodies may be used in

assays to monitor patients being treated with variant product, its activators, or its deactivators. Diagnostic assays for variant protein include methods utilizing the antibody and a label to detect variant product in human body fluids or extracts of cells or tissues. The products and antibodies of the present invention may be used with or without modification. Frequently, the proteins and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known in the art.

A variety of protocols for measuring the variant product, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescent activated cell sorting (FACS). As noted above, a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on variant product is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, *et al. (supra)*. Such protocols provide a basis for diagnosing altered or abnormal levels of variant product expression. Normal or standard values for variant product expression are established by combining body fluids or cell extracts taken from normal subjects, preferably human, with antibody to variant product under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by various methods, preferably by photometric methods. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by disease. Deviation between standard and subject values establishes the presence of disease state.

The antibody assays are useful to determine the level of variant product present in a body fluid sample, in order to determine whether it is being expressed at all, whether it is being overexpressed or underexpressed in the tissue, or as an indication of how variant levels of variable products are responding to drug treatment.

By another aspect the invention concerns methods for determining the presence or level of various anti-variant antibodies in a biological sample obtained from patients, such as blood or serum sample using as an antigen the variant product. Determination of said antibodies may be indicative to a plurality of pathological conditions or diseases.

C. Therapeutic uses of antibodies

In addition to their diagnostic use the antibodies may have a therapeutical utility in blocking or decreasing the activity of the variant product in pathological conditions where beneficial effect can be achieved by such a decrease.

The antibody employed is preferably a humanized monoclonal antibody, or a human Mab produced by known globulin-gene library methods. The antibody is administered typically as a sterile solution by IV injection, although other parenteral routes may be suitable. Typically, the antibody is administered in an amount between about 1-15 mg/kg body weight of the subject. Treatment is continued, e.g., with dosing every 1-7 days, until a therapeutic improvement is seen.